510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k042884

B. Purpose for Submission:
Marketing this test in the U.S.

C. Analyte:
Estrogen and Progesterone Receptors on formalin-fixed paraffin-embedded breast cancer specimens

D. Type of Test:
Immunohistochemical test for the semi-quantitative detection of estrogen and progesterone receptors

E. Applicant:
DakoCytomation California, Inc.

F. Proprietary and Established Names:
DakoCytomation ER/PR pharmDx™ Kit
Monoclonal Mouse Anti-Human Progesterone Receptor, Clone: PgR 1294

G. Regulatory Information:

1. Regulation section:
   21 CFR §864.1860 Immunohistochemistry reagents and kits
2. Classification:
   Class II
3. Product Code:
   MYA, MXZ
4. Panel:
   Pathology 88

H. Intended Use:

1. Intended use(s):

The DakoCytomation ER/PR pharmDx™ assay is a semi-quantitative immunohistochemical (IHC) kit system to identify estrogen receptor (ER) and progesterone receptor (PR) expression in normal and neoplastic tissues routinely processed and paraffin-embedded for histological evaluation. ER/PR pharmDx specifically detects the ER alpha protein as well as the PR protein located in the cell nucleus of ER and PR-expressing cells respectively.

The DakoCytomation Monoclonal Mouse Anti-Human Progesterone Receptor, clone PgR 1294, is intended for laboratory use as a semi-quantitative detection of progesterone receptor by light microscopy in routinely processed normal and pathological human paraffin-embedded tissue. This antibody is indicated for use as an aid in the management, prognosis and prediction of outcome of breast cancer.

2. Indication(s) for use:
   ER/PR pharmDx is indicated as an aid in identifying patients eligible for treatment with anti-hormonal or aromatase inhibitor therapies, as well as an aid in the prognosis and management of breast cancer.
3. Special condition for use statement(s):

4. Special instrument Requirements:
   The automated kit is for use with the Dako Autostainer.

I. Device Description:

The DakoCytomation ER/PR pharmDx™ assay is a semiquantitative immunohistochemical (IHC) kit system to identify estrogen receptor (ER) and progesterone receptor (PR) expression in normal and neoplastic tissues routinely processed and paraffin-embedded for histological expressing cells respectively. ER/PR pharmDx assay is available in two configurations, both manual and automated and is optimized for use with DakoCytomation detection systems. The kit contains enough materials to perform a maximum of 50 tests and 10 individual staining runs. It consists of the materials listed below:

DakoCytomation Monoclonal Mouse Anti-Human Estrogen Receptor Cocktail, clones 1D5 and ER-2-123. The ER cocktail specifically binds to estrogen receptor antigen located in the nuclear region of a variety of normal and neoplastic tissues. It contains monoclonal mouse IgG1 and IgG2a anti-human antibodies to human ER in tissue culture supernatant in a Tris-HCL buffer, pH 7.2, containing stabilizing protein and 0.015 mol/L sodium azide as preservative.

DakoCytomation Monoclonal Mouse Anti-Human Progesterone Receptor, clone PgR 1294. The PR antibody specifically binds to progesterone receptor antigen located in the nuclear region of a variety of normal and neoplastic tissues. It contains monoclonal IgG1 anti-human antibodies to human PR in tissue culture supernatant in a Tris-HCL buffer, pH 7.2, containing stabilizing protein and 0.015 mol/L sodium azide as preservative. It will also be available as a concentrate.

DakoCytomation Negative Control Reagent. It contains monoclonal mouse IgG1 and IgG2a antibody in tissue culture supernatant in a Tris-HCL buffer, containing stabilizing protein and 0.015 mol/L sodium azide.

Control Slides. Each slide contains sections of two pelleted, formalin-fixed, paraffin-embedded cell lines, which represent a moderate level of ER or PR protein expression (dependent on primary antibody applied to slide) and a negative cell line.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ER: Ventana ER, clone 6F11
   DakoCytomation ER, clone 1D5
   PR: Dako Cytomation PR, clone PgR 636
2. Predicate K number(s):
   K984567
   K993957
   K020023
3. Comparison with predicate:

Estrogen Receptor

Similarities		
Item	**Device**	**Predicate**
Intended Use	Semi-quantitative detection of estrogen receptor	Semi-quantitative detection of estrogen receptor
Antibody type	Monoclonal, mouse origin	Monoclonal, mouse origin
Technology	Immunohistochemistry	Immunohistochemistry
Tissue Type	Formalin-fixed, paraffin embedded breast tissue	Formalin-fixed, paraffin embedded breast tissue
Differences		
Item	**Device**	**Predicate**
Clone	1D5/ER-2-123	1D5 and 6F11
Interpretation of results	Allred Method; 3-8 = positive (>1-10% positive staining cells)	Positive = > 11% positive staining cells

Progesterone Receptor

Item	**Device**	**Predicate**
Intended Use	Semi-quantitative detection of progesterone receptor	Semi-quantitative detection of progesterone receptor
Antibody type	Monoclonal, mouse origin	Monoclonal, mouse origin
Technology	Immunohistochemistry	Immunohistochemistry
Tissue Type	Formalin-fixed, paraffin embedded breast tissue	Formalin-fixed, paraffin embedded breast tissue
Differences		

Item	Device	Predicate
Clone	PgR 1294	PgR 636
Interpretation of results	Allred Method; 3-8 = positive (>1-10% positive staining cells)	Positive = > 11% positive staining cells

K. Standard/Guidance Document Referenced (if applicable):

"In Vitro Diagnostic Devices: Guidance for the Preparation of 510(k) Submissions"
"Guidance for Industry: Guidance for Submission of Immunohistochemistry Applications to the FDA"

L. Test Principle:

The ER/PR pharmDx assay consists of a two-step immunohistochemistry staining procedure for routinely processed, paraffin-embedded specimens. Immunohistochemistry is a well established, widely accepted laboratory methodology. The assay detects the presence of Estrogen receptor (ER) and Progesterone receptor (PR) through first, the binding of an antibody to the antigen (ER or PR) of interest and second, visualization of the bound primary antibody through a reagent based on dextran technology. This reagent consists of both secondary goat anti-mouse antibody molecules and horseradish peroxidase molecules linked to a common dextran polymer backbone, thus eliminating the need for sequential application of link antibody and peroxidase conjugate. The enzymatic conversion of the subsequently added chromogen results in formation of a visible reaction product at the antigen site. The specimen is then counterstained and coverslipped. Results are interpreted using a light microscope. Control slides containing formalin-fixed, paraffin-embedded human cell lines are provided for quality control of the kit reagent performance.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
    a. *Precision/Reproducibility:*

Three paraffin embedded tissues representative of antigen expression in breast carcinoma were selected for staining. Eight serial sections from each tissue were prepared for a total of 24 slides. A range of expression levels was utilized to demonstrate reproducibility across sensitivity levels as represented by staining intensity. Staining intensity should no vary more than one grade within one tissue across the different staining.

Intra-run Reproducibility

Nine randomized tissue slides from 3 different tissue blocks containing breast carcinoma were stained during one procedure with the ER/PR pharmDx assay. Staining intensity among the 3 slides from each block was evaluated. The staining pattern was reported as nuclear. Background was reported as 0 and the slides tested with the negative reagent control were reported to have 0 staining. Staining

intensity was equivalent (within ½ grade) between the repeated staining of any of the three slides for each antibody.

Inter-run Reproducibility

The staining intensity across three days using the same reagents and the same tissues was compared for the ER/PR pharmDx assay. Testing was not performed on consecutive days. For each tissue staining intensity across the different days remained the same. The staining pattern was reported as nuclear, background was reported as 0, and the slides tested with the negative reagent control were reported to have 0 staining.

b. Linearity/assay reportable range:

N/A

c. Traceability (controls, calibrators, or method):

ER/PR pharmDx Control slides are supplied with the kit. Each of the control slides contains two pelleted, formalin-fixed, paraffin-embedded human cell lines, one that stains with ER and PR antibodies and one that does not stain. Two slides should be stained with each staining procedure, on with the anti-ER antibody cocktail and one with the anti-PR antibody. These slides are to be used to indicate the validity of the staining run. They should not be used as an aid in the interpretation of patient results. The pathologist is responsible for running additional positive and negative controls and assuring that the ER/PR pharmDx assay is performing properly.

d. Detection limit:

N/A

e. Analytical specificity:

A total of 90 formalin-fixed and paraffin-embedded tissues covering a wide range of normal human tissue types were tested with the ER/PR pharmDx assay. The antibodies demonstrated negative immunoreactivity with most tissues. Positive immunoreactivity was noted with some normal tissues which are typically positive, like uterus, ovary, and ductal epithelial cells of the breast.

f. Assay cut-off:

An Allred score of greater than 2 (corresponding to as few as 1% to 10% weakly positive cells) was used to define ER and PR positivity on the basis of a univariate cut-point analysis of all possible scores and disease-free survival (DFS) in patients receiving any adjuvant endocrine therapy. [1,2]

2. Comparison studies:

a. Method comparison with predicate device:

Immunoreactivity of the assay was tested on 210 tissues (75% ER+ and 25% ER-) and 204 (64% PR + and 36% PR-) assembled in tissue arrays. Testing consisted of staining the specimens using the Allred procedure (anti-ER clone 6F11 and anti-PR clone PgR 1294) compared to the ER/PR pharmDx procedure and evaluation of both

using the Allred scoring method. Slides for the Allred procedure were stained at Baylor College of Medicine, Houston, Tx. Slides for the ER/PR pharmDX assay were stained manually at DakoCytomation. Staining was assessed as a proportion score (PS) and an intensity score (IS) in the malignant tissue. Positive or negative status was determined based on the total Allred score (PS + IS > 2 = positive). Sensitivity of the ER test method was 98.75% while the specificity was 100%. Sensitivity of the PR test method was 98.46% while the specificity was 100%. Results, in 2 X 2 tables below, reveal concordance of the ER/PR pharmDx assay to the Allred method for positive/negative hormone receptor of 99% for each receptor.

2 x 2 table of DakoCytomation ER test results compared to Allred Procedure ER test result

	Allred Procedure ER Test Result		
DAKO Cytomation ER test result	Positive n	Negative n	Total N (%)
Positive	158	0	158 (74.5%)
Negative	2	52	54 (25.5)
Total	160	52	212

2 x 2 table of DakoCytomation PR test results compared to Allred Procedure PR test result

	Allred Procedure PR Test Result		
DAKO Cytomation PR test result	Positive n	Negative n	Total N (%)
Positive	128	0	128 (62.7%)
Negative	2	74	54 (37.3)
Total	130	74	204

Additional published studies compare the Allred procedure (anti-ER clone 6F11 and anti-PR clone PgR 1294) and Ligand Binding Assay

(LBA), a well-established reference methodology and the capability of each method to predict disease free survival.[1,2] The ER study evaluated 1,982 primary breast cancers while the PR study evaluated 1,294 primary breast cancers. ER and PR positivity was scored by the Allred scoring method (0-8) and compared to LBA (with a cut-off positivity value of 3 and 5 fmol/mg for ER and PR, respectively). Each method was then compared to clinical outcome.

An Allred score of greater than 2 (corresponding to as few as 1% to 10% weakly positive cells) was used to define ER and PR positivity on the basis of a univariate cut-point analysis of all possible scores and disease-free survival (DFS) in patients receiving any adjuvant endocrine therapy. Using the Allred method, 70.5% of all tumors were determined to be ER positive compared with 78.9% by LBA. Also by the Allred method, 54% of tumors were determined to be PR positive compared to 56% by LBA. The concordance of the ER and PR to the LBA was 85.5% and 86%, respectively. Among patients who received adjuvant endocrine therapy, ER and PR was a strong predictor of both disease-free and overall survival. The best cutoff for both end points was a total score >2.

The concordance of anti-PR clone PgR 1294, anti-PR clone PgR 636, and Dextran Coated Charcoal (DCC) in 30 breast cancer tissues was also tested. Immunostaining was considered positive when at least 5% of the nuclei from three randomly selected fields a section were stained. Specimens were considered PR positive by the biochemical DCC assay when progestin binding values were ≥10 fmol/mg. Of the 30 specimens evaluated by IHC, 76.7% (23/30) were positive with clone PgR 1294. These results were 100% concordant with clone PgR 636. When clone PgR 1294 was compared to the DCC assay, concordance was 90% (27/30).

[1] Harvey JM, Clark, GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 1999; 17:1474

[2] Mohsin SK, Weiss H, Havighurst T, Clark GM, Berardo M, Roanh LD, To TV, Love RL, Allred DC. Progesterone receptor by immunohistochemistry and clincal outcome in breast cancer: a validation study. Mod Pathol 2004;17:1545

b. Matrix comparison:

3. Clinical studies:
   a. Clinical sensitivity:
   N/A

b. Clinical specificity:
N/A
c. Other clinical supportive data (when a and b are not applicable):

4. Clinical cut-off:
N/A
5. Expected values/Reference range:
N/A

N. Conclusion:

The submitted material in this premarket notification is complete and support a substantial equivalence decision.